PATENT
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Applicants:	Palsson et al.	Art Unit:	1631
Serial No.:	10/087,441	Examiner	R. S. Negin
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Title:	MODELS AND METHODS FOR DETERMINING SYSTEMIC PROPERTIES OF REGULATED REACTION NETWORKS		

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RESPONSE TO THE OFFICE ACTION

Sir:

Responsive to the Final Office Action dated April 7, 2011, entry of the amendments and consideration of the Remarks below is respectfully requested.

The current listing of claims begins on page **2** of this paper

Remarks begin on page **13** of this paper.

CURRENT LISTING OF CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

1. (currently amended) A computer readable medium or media having stored thereon computer-implemented instructions causing a processor to perform the steps of:

(a) providing a data structure comprising a stoichiometric matrix relating a plurality of reactants to a plurality of reactions of a biochemical reaction network of an organism, wherein each of said reactions comprises a reactant identified as a substrate of the reaction, a reactant identified as a product of the reaction and a stoichiometric coefficient relating quantity of said substrate and quantity of said product in a reaction, and wherein at least one of said reactions is a regulated reaction;

(b) providing a constraint set for said plurality of reactions of said organism, wherein said constraint set comprises a variable constraint for said regulated reaction,

(c) employing flux balance analysis to solve a mathematical optimization problem using said stoichiometric matrix by determining at least one flux distribution that minimizes or maximizes an objective function when said constraint set is applied to said data structure, wherein said at least one flux distribution determines a systemic property predictive of said biochemical reaction network of said organism, and wherein said systemic property is dependent upon the flux through said regulated reaction, and

(d) providing information resulting from steps (a) through (c) to a user.

2. (original) The computer readable medium or media of claim 1, wherein said variable constraint is dependent upon the outcome of at least one reaction in said data structure.

3. (original) The computer readable medium or media of claim 1, wherein said variable constraint is dependent upon the outcome of a regulatory event.

4. (original) The computer readable medium or media of claim 1, wherein said variable constraint is dependent upon time.

5. (original) The computer readable medium or media of claim 1, wherein said variable constraint is dependent upon the presence of a biochemical reaction network participant.

6. (original) The computer readable medium or media of claim 5, wherein said participant is selected from the group consisting of a substrate, product, reaction, protein, macromolecule, enzyme and gene.

7. (original) The computer readable medium or media of claim 1, wherein said biochemical reaction network comprises metabolic reactions.

8. (original) The computer readable medium or media of claim 1, further comprising a regulatory data structure, wherein said variable constraint is dependent upon an outcome of a regulatory event represented by said regulatory data structure.

9. (original) The computer readable medium or media of claim 8, wherein said regulatory data structure represents a regulatory event selected from the group consisting of transcription of a gene, translation of an RNA, post-translational modification of a protein, inhibition of a protein, activation of a protein, assembly of a protein, change in pH, change in redox potential, change in temperature, passage of time, and degradation of a protein.

10. (original) The computer readable medium or media of claim 8, wherein said regulatory event is due to a signal transduction pathway.

11. (original) The computer readable medium or media of claim 8, wherein said biochemical reaction network and said regulatory data structure represent reactions or events that occur in a single cell.

12. (original) The computer readable medium or media of claim 8, wherein said biochemical reaction network represents reactions that occur in a first cell in a population of cells and said regulatory data structure represents events that occur in a second cell in said population.

13. (original) The computer readable medium or media of claim 12, wherein said population of cells comprises cells of a multicellular organism.

14. (original) The computer readable medium or media of claim 1, further comprising a constraint function that correlates an outcome of a regulatory event with said variable constraint.

15. (original) The computer readable medium or media of claim 14, wherein said constraint function is binary.

16. (original) The computer readable medium or media of claim 14, wherein said regulatory event is represented by Boolean logic.

17. (cancelled)

18. (previously presented) The computer readable medium or media of claim 1, further comprising a range of said feasible flux distributions.

19. (previously presented) The computer readable medium or media of claim 1, wherein said commands comprise an optimization problem.

20. (original) The computer readable medium or media of claim 19, wherein said optimization problem comprises a linear optimization problem or a nonlinear optimization problem.

21. (previously presented) The computer readable medium or media of claim 1, further comprising a user interface capable of sending at least one command for modifying said data structure, said constraint set or said commands for applying said constraint set to said data representation, or a combination thereof.

22. (original) The computer readable medium or media of claim 21, wherein said user interface further comprises links which a user may select to access additional information relating to said plurality of reactions.

23. (original) The computer readable medium or media of claim 1, wherein said data structure comprises a set of linear algebraic equations.

24. (original) The computer readable medium or media of claim 1, wherein said data structure comprises a matrix.

25. (original) The computer readable medium or media of claim 1, further comprising commands for representing said at least one flux distribution as a flux distribution map.

26. (original) The computer readable medium or media of claim 1, wherein at least one reactant in said plurality of reactants or at least one reaction in said plurality of reactions is annotated.

27. (original) The computer readable medium or media of claim 26, wherein said annotation comprises assignment of said at least one reactant to a compartment.

28. (original) The computer readable medium or media of claim 27, wherein a first substrate or product in said plurality of reactions is assigned to a first compartment and a second substrate or product in said plurality of reactions is assigned to a second compartment.

29. (original) The computer readable medium or media of claim 26, wherein said annotation comprises assignment to an open reading frame or protein.

30. (previously presented) The computer readable medium or media of claim 26, wherein said annotation comprises a confidence limit for occurrence of a reaction.

31. (original) The computer readable medium or media of claim 1, further comprising a gene database relating one or more reactions in said data structure with one or more genes or proteins in particular organism.

32. (original) The computer readable medium or media of claim 1, wherein said biochemical reaction network comprises reactions that are selected from the group consisting of glycolysis, the TCA cycle, the pentose phosphate pathway, respiration, biosynthesis of an amino acid, degradation of an amino acid, biosynthesis of a purine, biosynthesis of a pyrimidine, biosynthesis of a lipid, metabolism of a fatty acid, biosynthesis of a cofactor, metabolism of a cell wall component, transport of a metabolite and metabolism of carbon, nitrogen, sulfur, phosphate, hydrogen or oxygen.

33. (original) The computer readable medium or media of claim 1, wherein a plurality of said reactions are regulated reactions and said constraints for said regulated reactions comprise variable constraints.

34. (currently amended) A method for determining a systemic property of a biochemical reaction network of an organism, comprising:

(a) providing a data structure comprising a stoichiometric matrix relating a plurality of reactants to a plurality of reactions of a biochemical reaction network of an organism,

wherein each of said reactions comprises a reactant identified as a substrate of the reaction, a reactant identified as a product of the reaction and a stoichiometric coefficient relating quantity of said substrate and quantity of said product in a reaction, and wherein at least one of said reactions is a regulated reaction;

(b) providing a constraint set for said plurality of reactions of said organism,

wherein said constraint set comprises a variable constraint for said regulated reaction;

(c) providing a condition-dependent value to said variable constraint;

(d) providing an objective function;

(e) employing flux balance analysis to solve a mathematical optimization problem using said stoichiometric matrix by determining on a computer at least one flux distribution that

minimizes or maximizes said objective function when said constraint set is applied to said data structure,

wherein said at least one flux distribution is determinative of a systemic property predictive of said biochemical reaction network of said organism, and

(f) providing said systemic property of said biochemical reaction network to a user.

35. (previously presented) The method of claim 34, wherein said condition-dependent value provided to said variable constraint comprises a value conditioned on the outcome of at least one reaction in said data structure.

36. (previously presented) The method of claim 34, wherein said condition-dependent value provided to said variable constraint comprises a value conditioned on the outcome of a regulatory event.

37. (previously presented) The method of claim 34, wherein said condition-dependent value provided to said variable constraint comprises a value conditioned on time.

38. (previously presented) The method of claim 34, wherein said condition-dependent value provided to said variable constraint comprises a value conditioned on the presence of a biochemical reaction network participant.

39. (original) The method of claim 38, wherein said participant is selected from the group consisting of a substrate, product, reaction, enzyme, protein, macromolecule and gene.

40. (original) The method of claim 34, wherein said biochemical reaction network comprises metabolic reactions.

41. (previously presented) The method of claim 34, wherein said data structure further comprises a regulatory data structure, wherein said value provided to said variable constraint is changed due to an outcome of a regulatory event represented by said regulatory data structure.

42. (original) The method of claim 41, wherein said regulatory event is selected from the group consisting of transcription of a gene, translation of an RNA, post-translational modification of a protein, inhibition of a protein, activation of a protein, assembly of a protein, change in pH, change in redox potential, change in temperature, passage of time, and degradation of a protein.

43. (original) The method of claim 41, wherein said regulatory event is due to a signal transduction pathway.

44. (original) The method of claim 41, wherein said biochemical reaction network and said regulatory data structure represent reactions or events that occur in a single cell.

45. (original) The method of claim 41, wherein said regulatory event comprises a regulatory reaction.

46. (original) The method of claim 41, wherein said biochemical reaction network represents reactions that occur in a first cell in a population of cells and said regulatory data structure represents events that occur in a second cell in said population.

47. (original) The method of claim 46, wherein said population of cells comprises cells of a multicellular organism.

48. (original) The method of claim 41, further comprising a constraint function that correlates an outcome of a regulatory event with said variable constraint.

49. (original) The method of claim 48, wherein said constraint function is binary.

50. (original) The method of claim 48, wherein said regulatory event is represented by Boolean logic.

51. (original) The method of claim 48, wherein said constraint function correlates a first set of outcomes of said regulatory data structure with a first binary value and a second set of outcomes of said regulatory data structure with a second binary value.

52. (original) The method of claim 48, wherein said constraint function correlates a set of outcomes of said regulatory data structure with a single integer value.

53. (original) The method of claim 34, wherein said flux distribution is determined by optimization.

54. (original) The method of claim 53, wherein said optimization comprises linear optimization or non linear optimization.

55. (original) The method of claim 34, further comprising a step of modifying said data structure or said constraint set, or both.

56. (original) The method of claim 34, wherein said data structure comprises a set of linear algebraic equations.

57. (original) The method of claim 34, wherein said data structure comprises a matrix.

58. (original) The method of claim 34, further comprising a step of producing a flux distribution map.

59. (original) The method of claim 34, wherein said biochemical reaction network comprises reactions that are selected from the group consisting of glycolysis, the TCA cycle, pentose phosphate pathway, respiration, biosynthesis of an amino acid, degradation of an amino acid, biosynthesis of a purine, biosynthesis of a pyrimidine, biosynthesis of a lipid, metabolism of a fatty acid, biosynthesis of a cofactor, metabolism of a cell wall component, transport of a metabolite and metabolism of a carbon source, nitrogen source, oxygen source, phosphate source, hydrogen source or sulfur source.

60. (original) The method of claim 34, wherein said systemic property is selected from the group consisting of growth, energy production, redox equivalent production, biomass production, production of biomass precursors, production of a protein, production of an amino acid, production of a purine, production of a pyrimidine, production of a lipid, production of a fatty acid, production of a cofactor, production of a cell wall component, transport of a metabolite, development, intercellular signaling, and consumption of carbon nitrogen, sulfur, phosphate, hydrogen or oxygen.

61. (original) The method of claim 34, wherein said systemic property is selected from the group consisting of degradation of a protein, degradation of an amino acid, degradation of a purine, degradation of a pyrimidine, degradation of a lipid, degradation of a fatty acid, degradation of a cofactor and degradation of a cell wall component.

62. (original) The method of claim 34, wherein said variable constraint comprises a condition-dependent constraint value and a constraint function, wherein said variable constraint is modified by said constraint function acting upon said condition-dependent constraint value.

63. (original) The method of claim 62, wherein said constraint function is binary.

64. (original) The method of claim 34, further comprising providing a gene database relating one or more reactions in said data structure with one or more open reading frames or proteins in a particular organism.

65. (original) The method of claim 64, further comprising identifying an open reading frame that encodes a protein that performs a reaction in said plurality of reactions.

66. (original) The method of claim 64, further comprising identifying a protein that performs a reaction in said plurality of reactions.

67. (withdrawn) A method for determining a phenotype of a mutant of an organism, comprising:

(i) identifying a reaction that is not naturally present in a particular organism, and

(ii) determining a systemic property of a biochemical reaction network according to the method of claim 34, wherein said data structure relates a plurality of reactants for said organism to a plurality of reactions of a biochemical reaction network of said organism and further comprises said reaction that is not naturally present in said organism.

68. (withdrawn) A method for determining a phenotype of a mutant of an organism, comprising:

(i) identifying a reaction that is related to an open reading frame or protein in said gene database, and

(ii) determining a systemic property of a biochemical reaction network according to the method of claim 34, wherein said reaction that is related to said open reading frame or protein is not present in said data structure or is constrained to have no flux.

69. (withdrawn) A method for determining the effect of a drug on the activity of one or more reactions in a biochemical reaction network, comprising:

(i) identifying a reaction that is related to an open reading frame or protein in said gene database;

(ii) identifying a candidate drug that alters expression of said open reading frame or activity of said protein, and

(iii) determining a systemic property of a biochemical reaction network according to the method of claim 34, wherein said reaction that is related to said open reading frame or protein is not present in said data structure, is constrained to have a reduced flux, or is constrained to have no flux.

70. (original) The method of claim 34, wherein a plurality of said reactions are regulated reactions and said constraints for said regulated reactions comprise variable boundary values.

71. (currently amended) A method for determining a systemic property of a biochemical reaction network at a first and second time, comprising:

(a) providing a data structure comprising a stoichiometric matrix relating a plurality of reactants to a plurality of reactions of a biochemical reaction network of an organism,

wherein each of said reactions comprises a reactant identified as a substrate of the reaction, a reactant identified as a product of the reaction and a stoichiometric coefficient relating quantity of said substrate and quantity of said product in a reaction, and wherein at least one of said reactions is a regulated reaction;

(b) providing a constraint set for said plurality of reactions of said organism, wherein said constraint set comprises a variable constraint for said regulated reaction;

(c) providing a condition-dependent value to said variable constraint;

(d) providing an objective function;

(e) employing flux balance analysis to solve a mathematical optimization problem using said stoichiometric matrix by determining on a computer at least one flux distribution at a first time that minimizes or maximizes said objective function when said constraint set is applied to said data structure,

thereby determining a systemic property predictive of said biochemical reaction network of said organism at said first time;

(f) modifying said value provided to said variable constraint;

(g) repeating step (e) wherein said at least one flux distribution is determined at a second time, thereby determining a systemic property predictive of said biochemical reaction network of said organism at a second time, and

(h) providing said systemic property of said biochemical reaction network to a user at said first, second or first and second time.

72. (original) The method of claim 71, wherein said value is modified based on said flux distribution at said first time.

73. (original) The method of claim 71, wherein said value is modified based on a change in an environmental condition.

REMARKS

Claims 1-16 and 18-74 are currently pending. Claims 67-69 stand withdrawn from consideration as being directed to a non-elected invention. Applicants reserve the right to pursue these claims in a later filed application claiming the benefit of the subject application. Claims 1-16, 18-66 and 70-74 are under examination and claims 1, 34 and 71 have been amended. Support for the amendment can be found throughout the application as filed, including for example, at paragraph 0041. Accordingly, these amendments do not raise an issue of new matter, and entry thereof is respectfully requested.

Applicants submit herewith a Supplemental Information Disclosure Statement with copies of the omitted references referred to at page 3 of the Office Action mailed .

Rejections Under 35 U.S.C. § 103

Claims 1-12, 14, 15, 18-28, 30, 31-46, 48-49, 51-63 and 70-74 stand rejected under 35 U.S.C. § 103(a) as allegedly obvious over Hatzimanikatis et al., *AICHE Journal* 42:1277-1292 (1996), in view of Varma et al., *Bio/Technology* 12:994-998 (1994) and further in view of Grewal et al., *Protein Engineering* 7:205-211 (1994).

Applicants respectfully traverse and point out that the cited combination of references or general knowledge in the art fail to suggest or provide an incentive to one skilled in the art to arrive at the claimed invention with a reasonable expectation of success.

The Examiner appears to maintain the above rejection on two general points. First, that both Hatzimanikatis et al. and Varma et al. employ or suggest stoichiometric matrices and flux balance analysis to solve reaction network optimization and, second, that the binary term of Hatzimanikatis et al. is similar to the claimed stoichiometric coefficient. Office Action, pp.16-20.

In providing one rationale under the first point above, the Examiner characterizes Applicants' arguments of record to be that Hatzimanikatis et al. is not directed to kinetics and

that the flux balance analyses of Varma et al. reflect a kinetic analysis. Office Action, p. 16, para. 1. Applicants respectfully point out that this characterization is exactly the opposite of Applicants' arguments of record. See, e.g., Responses filed May 21, 2010, and August 2, 2010. Hatzimanikatis et al. employ a kinetic analysis and Varma et al. employ flux balance analysis. As described in detail in previous Responses and in Exhibit A to the response filed on May 21, 2010, these two computational procedures are distinct. In addition, the Examiner states:

> [C]laim 1 is conducting both mass balances (step a) in combination with kinetics [*sic*] analysis that are based on the same stoichiometric matrices (step c).

Id.

This characterization also is opposite of Applicants' arguments of record. Claim 1 explicitly recites "employing flux balance analysis," which is a computation al procedure distinct from the kinetic analysis of Hatzimanikatis et al. See, e.g., Response filed May 21, 2010.

With respect to the second point above, Applicants have amended the claim to more explicitly point out that the claimed stoichiometric coefficient relates a quantity of a substrate with a quantity of a product in a reaction. As pointed out of record, the claimed stoichiometric coefficient is distinct and non-analogous to the binary term of Hatzimanikatis et al. because the claimed stoichiometric coefficient applies to substrates and products whereas the binary term of Hatzimanikatis et al. applies to reactions. Further, the claimed stoichiometric coefficient relates the quantity of a substrate with the quantity of a product in a reaction whereas the binary term merely indicates the presence or absence of a reaction. A stoichiometric coefficient of zero, as hypothesized in the Office Action (see, e.g., p. 17, para. 3), is nonsensical because the chemical equation employed in the claimed stoichiometric matrix would be chemically incorrect as it would not be balanced. In addition, the claimed stoichiometric coefficient does not indicate a flux as alleged in the Office Action (see, e.g., page 18, para. 1). Rather, the claims recite solving a mathematical optimization problem by flux balance analysis to determine flux distribution.

As Applicants' point out above and previously of record, the primary and secondary references, together with knowledge generally known in the art, would not lead one of ordinary

skill in the art to arrive at the claimed invention because the computational approaches are distinct and non-analogous. Although cited with respect to claim 1, Grewal et al. is applied to dependent claims 12 and 46 in the body of the Office Action and appears to be directed to signal transduction pathways. Such teachings fails to cure the deficiencies in the combination of the primary and secondary references. Accordingly, Applicants respectfully request that this ground of rejection be withdrawn.

Claims 31 and 64-66 stand rejected under 35 U.S.C. § 1039(a) as allegedly obvious over Hatzimanikatis et al., *supra*, in view of Varma et al., *supra*, and Grewal et al., *supra*, and further in view of Liao et al., *Biotechnol. Bioengineer.* 52:129-140 (1996). Applicants respectfully traverse. This rejection relies on Hatzimanikatis et al. in view of Varma et al. and, as discussed above, Applicants have set forth the deficiencies of Hatzimanikatis et al. in view of Varma et al. and Grewal et al., and Liao et al. does not cure these deficiencies. Accordingly, the claimed methods are unobvious over the cited combination of references and withdrawal of this ground of rejection is respectfully requested.

Claims 16 and 50 stands rejected under 35 U.S.C. § 103(a) as allegedly obvious over Hatzimanikatis et al., *supra*, in view of Varma et al., *supra*, and Grewal et al, and further in view of Kim et al., U.S. publication 2002/00087275. Applicants respectfully traverse. This rejection relies on Hatzimanikatis et al. in view of Varma et al., and Applicants have set forth above the deficiencies in the combination of these primary and secondary references. Kim et al. does not cure these deficiencies alone or in combination with Grewal et al. Accordingly, the claimed method is unobvious over the cited combination of references and withdrawal of these ground of rejection is respectfully requested.

Claims 13 and 47 stand rejected under 35 U.S.C. § 103(a) as allegedly obvious over Hatzimanikatis et al., *supra*, in view of Varma et al., *supra*, and Grewal et al., *supra*, and further in view of Vissing et al., *Neurology* 47:766-771 (1996). Applicants respectfully traverse. This rejection relies on Hatzimanikatis et al. in view of Varma et al., and Applicants have set forth above the deficiencies in the combination of these primary and secondary references Vissing et

al. does not cure these deficiencies alone or in combination with Grewal et al. Accordingly, the claimed computer readable medium or media and method are unobvious over the cited combination of references and withdrawal of this ground of rejection is respectfully requested.

Claim 29 stands rejected under 35 U.S.C. § 103(a) as allegedly obvious over Hatzimanikatis et al., *supra*, in view of Varma et al., *supra*, and Grewal et al., *supra*, and further in view of Callis, *Plant Cell* 7:845-857 (1995). Applicants respectfully traverse. As discussed above, Applicants have set forth the deficiencies of Hatzimanikatis et al. in view of Varma et al. and/or Grewal et al. Moreover, Applicants respectfully submit that Callis does not cure the deficiencies of this combination of references. As set forth previously of record, Callis is a review article discussing regulation of protein degradation in plants. Furthermore, Applicants respectfully submit that the passage on page 850 of Callis referred to in the Office Action describes the senescent process in unpollinated pea ovaries and the induction of a cysteine protease during this process. There is no teaching or suggestion of annotation of at least one reactant in a plurality of reactants or at least one reaction in a plurality of reactions by assignment to an open reading frame, as in Applicants' claim. Applicants respectfully maintain that Callis provides no motivation as asserted in the Office Action. Therefore, Applicants respectfully submit that Callis does not cure the deficiencies of Hatzimanikatis et al. in view of Varma et al. and/or Grewal et al. Accordingly, the claimed computer readable medium or media is unobvious over the cited combination of references and withdrawal of this ground of rejection is respectfully requested.

CONCLUSION

In light of the amendments and Remarks herein, Applicants submit that the claims are in condition for allowance and respectfully requests a notice to this effect. Should the Examiner have any questions, he is invited to call the undersigned attorney.

The Commissioner is hereby authorized to charge $1100.00 as payment for the Petition for the Three-Month Extension of Time fee and the RCE fee to Deposit Account No. 07-1896. Additionally, the Commissioner is hereby authorized to charge any other fees that may be due in connection with the filing of this paper, or credit any overpayment to Deposit Account No. 07-1896.

Respectfully submitted,

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